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INFLUENCE OF RIFFLE AND SNAG HABITAT SPECIFIC SAMPLING ON STREAM MACROINVERTEBRATE ASSEMBLAGE MEASURES IN BIOASSESSMENT

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Abstract. Stream macroinvertebrate communities vary naturally among types of habitats where they are sampled, which affects the results of environmental assessment. We analyzed macroinvertebrates collected from riffle and snag habitats to evaluate influences of habitat-specific sampling on taxon occurrence, assemblage measures, and biotic indices. We found considerably more macroinvertebrate taxa unique to snags (143 taxa) than to riffles (75 taxa), and the numbers of taxa found in both riffles and snags (149 taxa) were similar to that found in snags. About 64% of the 47 macroinvertebrate measures we tested differed significantly between riffles and snags. Eighty percent intercepts of regressions between biotic indices and urban or agricultural land uses differed significantly between riffles and snags. The Hilsenhoff biotic index calculated from snag samples explained 69% of the variance of riffle samples and classified 66% of the sites into the same stream health group as the riffle samples. However, four multimetric indices for snag samples explained less than 50% of the variance of riffle samples and classified less than 50% of the sites into the same health group as the riffle samples. We concluded that macroinvertebrate indices developed for riffle/run habitat should not be used for snag samples to assess stream impairment. We recommend developing an index of biotic integrity specifically for snags and using snags as an alternate sampling substrate for streams that naturally lack riffles.

Keywords: macroinvertebrate, streams, bias, riffle habitat, snag habitat, macroinvertebrate biotic index, environmental impacts, habitat-specific sampling

1. Introduction

Stream benthic macroinvertebrates are widely used as indicators for assessing environmental impairment in North America and many other parts of the world. Macroinvertebrate community compositions are known to vary naturally in both spatial and temporal scales (e.g., Hilsenhoff, 1988; Merritt and Cummins, 1996; Li *et al.*, 2001), which potentially limits the ability of assessments to detect impairment. To overcome the influence of natural variability, comparisons are often restricted to areas with equivalent natural environmental characteristics (e.g., stratifying comparison within the same ecoregion or subecoregion; Weigel, 2003), or the same season (e.g., fall vs. spring; Hilsenhoff, 1988; Gibson *et al.*, 1996).

The type of habitat where macroinvertebrates are collected may also obscure results of environmental assessment. Two types of protocols are widely used to sample macroinvertebrates from natural habitat. One type of protocols emphasizes sampling a single habitat to standardize assessments among streams. This protocol was proposed by Hilsenhoff (1988) to collect macroinvertebrates from riffles with a current velocity greater than 3 m/s. Other examples include the original version of the rapid bioassessment protocols (RBPs) for use in streams and rivers (Plafkin *et al.*, 1989), and the national water-quality assessment program (NAWQA) protocol for macroinvertebrates (Cuffney *et al.*, 1993; Moulton *et al.*, 2002). The strength of sampling riffle habitat is the minimization of potential effects of inter-habitat variation. The weakness is that it can not be applied in low gradient streams where riffle habitat is naturally absent. The RBPs and NAWQA protocols recommend that in streams where riffle/run habitats are not available, other habitats such as vegetated banks, submerged macrophytes, woody debris, bridge abutments, pier pilings, and manufactured bed be sampled instead. Consequently, comparisons among sites with and without riffle/run habitats may be confounded by inter-habitat variation, which may obscure the assessment.

The other type of protocols is sampling multiple habitats. This approach is presented in the revised RBPs (Barbour *et al.*, 1997), which recommends sampling major habitats in proportion to their occurrence within a sampling reach for streams where cobble substrate represents less than 30% in the reference streams. Macroinvertebrates are collected from multiple habitats, such as coarse substrates, woody snags, vegetated banks, submerged macrophytes, and sand and silt sediments and are composited into a single sample. This approach is also used by the British Institute of Freshwater Ecology's River Invertebrate Prediction and Classification Scheme (Wright *et al.*, 1984), where macroinvertebrates are collected from all major habitats of a site, in proportion to their occurrence, to generate a composited sample. One strength of this approach is that it is applicable to streams with and without rocky substrate and is well suitable for classifying sites according to their macroinvertebrate taxon occurrence for conservation purpose. The weakness of using multi-habitat sampling for environmental assessment is that the taxa collected from a site may be weighted to the spatially dominant habitat type, and streams are assessed according to the particular habitat type represented rather than water quality or general environmental health (Parsons and Norris, 1996). Therefore, in making comparisons among streams, multi-habitat samples may introduce inter-habitat variation that can potentially mask water quality differences among sites.

Despite the weaknesses associated with single and multi-habitat sampling approaches, both methods have been widely used by water resource agencies and research institutes to assess environmental quality. Thus, it is important that inter-habitat variation is recognized and distinguished from environmental impairment when comparing sites using samples from different habitats.

Although differences in macroinvertebrate composition between riffle and pool habitats or among different rocky substrate sizes have been documented (e.g., McCulloch, 1986; Quinn and Hickey, 1990; Brown and Brussock, 1991), only limited studies have evaluated differences in macroinvertebrate collections between riffle and snag habitats. More studies are especially needed to evaluate the influences of such different habitats on the macroinvertebrate biotic indices.

In this study, we compared macroinvertebrate communities between riffle and snag habitats. Snag habitat consisted of coarse woody debris, logs, submerged macrophytes, overhanging vegetation, and leaf packs and other coarse organic materials accumulated on objects in the flowing water. Our overall goal was to investigate the effects of habitat-specific macroinvertebrate sampling on stream environmental assessment and to better understand where and how biases of habitat-specific sampling influence environmental assessments. Specifically, we asked whether or not macroinvertebrate taxa, assemblage measures, and indices of biotic integrity are substantially different between riffle and snag habitats. If yes, we want to know (1) the magnitude and nature of the differences between the two habitats, (2) if macroinvertebrate biotic indices developed for riffle habitat can be used for snag habitat to effectively assess environmental degradation, (3) if sampling snag habitat alone can be sufficient to assess environmental degradation in streams lacking of riffles, and (4) if our results from streams with riffles are applicable to streams without riffles.

2. Methods

2.1. Study sites

Data were collected from 142 sites on 1st to 4th order streams across Wisconsin and southeastern Minnesota, USA (Figure 1). Sites were selected to be easily accessible, permit a sampling of both riffle and snag habitats, represent a range of anthropogenic influence, and cover a range of natural variation in stream and catchment characteristics.

The study area has a range of landscape and local conditions. Forest and wetland dominate the catchments of the low-gradient systems in the north; a mixture of agriculture and woodland typifies the landscape of mixed gradient streams in the central; and urban and agriculture are the characteristics of the low gradient streams in the southeastern Minnesota and Wisconsin. By contrast, hilly land with forested hills and agricultural valleys of varied gradient systems are characteristics of the southwestern study area. The study catchment size varies from <5 to $>150\,km^2$ (mean = 30). The combination of woodland, wetland, and water ranges from <2 to 100% of the catchment. Agricultural land ranges from 0 to 90% (mean = 46%), and urban land varies from 0 to 93% (mean = 10%). Stream bottom gradient varies from near 0 to 18 m/km (mean = 4). Stream cobble and gravel substrates vary

Figure 1. Map of Wisconsin and Minnesota showing the sampling locations.

from <1 to 91% (mean = 48), and sand/silt substrates vary from <10 to >90% (mean = 44%). These ranges of conditions provided an ideal setting to test our hypothesis.

2.2. Catchment land-cover gathering and site-habitat sampling

Catchment boundaries upstream of each site were delineated using ArcView WATERSHED Avenue Command (ESRI, 1999) and a Digital Elevation Model with a 30-m resolution, and were verified and corrected using 1:24,000 digital topographic maps by referencing elevation contour lines. Land cover within each catchment was quantified using ARC/INFO software to overlay catchment boundaries on the land census databases. The data source for Wisconsin is Wisconsin Initiative for Statewide Cooperation on Landscape Analysis and Data digital land-cover map, which was generated from 30-m ground resolution data collected in 1992 (Lillesand *et al.*, 1998). The data source for Minnesota is Minnesota Land Use and Cover: 1990's Census of the Land (http://lucy.lmic.state.mn.us/metadata/luse8.html).

Stream physical habitat and conductivity were measured once between 1997 and 2000. We assessed physical habitat at a site length of 35 times the mean stream width, or a minimum of 100 m. This length was sufficient to encompass about three meander sequences and is commonly used for fish habitat assessment (Simonson *et al.*, 1994; Wang *et al.*, 1996). We sampled physical habitat between early June and mid-August when low stream flows facilitated effective sampling. At each site, 30 habitat variables, including channel morphology, bottom substrates, cover, bank conditions, riparian vegetation, and land cover were measured or visually estimated along 12 transects using standardized procedures (Simonson *et al.*, 1994). Additionally, we measured water conductivity using a YSI oxygen-conductivity meter (model 85) at the downstream end of each site before sampling physical habitats, because conductivity is an indicator that is easy to measure and strongly correlated with agricultural and urban land uses (Wang L. unpublished data).

2.3. Macroinvertebrate sampling

Two semi-quantitative samples were collected from each site, one from riffles and one from snags, using a 600 μm mesh D-frame kick net. Sampling occurred in early October at base-flow conditions between 1997 and 2000. Riffle samples were collected from cobble-gravel substrates following Hilsenhoff's (1988) procedures. A net was placed on the stream bottom and macroinvertebrates were dislodged immediately upstream of the net by kicking the substrate. This process was repeated in at least three locations within the same or different riffles for a total of five minutes for each site.

Snag samples were collected from coarse woody debris, logs, submerged macrophytes, overhanging vegetation, and leaf packs and other coarse organic materials accumulated on objects in the flowing water and were composited into a single sample. All available snag types at multiple locations of each site were sampled roughly in proportion to their occurrence with priority given to larger snags in areas of higher water velocity for a total of 10 minutes for each site.

In the laboratory, samples were placed in a glass pan positioned over a grid comprising 6.5 cm^2 squares. Grid squares were randomly chosen to separate macroinvertebrates from other materials until a minimum of 125 individuals with pollution tolerance values (judged by the laboratory technician's experience) were picked (Hilsenhoff, 1988). All other organisms without tolerance values in the selected grid squares were also picked. All picked organisms were enumerated and mostly identified to species.

2.4. Data summary

We summarized the 31 catchment land-use types into agriculture, grassland, urban, water, wetland, and woodland. Each category was expressed as a percentage of total catchment area. From the habitat data, we calculated summary statistics for variables that had been shown to influence macroinvertebrate composition (e.g., Merritt and Cummins, 1996), including substrate composition, channel morphology, and riparian land use.

From the macroinvertebrate data, we calculated 46 summary measures (functional groups are from Merritt and Cummins, 1996) that are commonly used for evaluating stream health (e.g., Ohio EPA, 1988; Kerans and Karr, 1994; Barbour *et al.*, 1997; Weigel, 2003) for riffle and snag samples separately in each site. Additionally, we calculated five macroinvertebrate biotic indices that have been widely used for assessing environmental impairment. The Hilsenhoff biotic index (HBI) is an abundance-weighted tolerance index calculated based on the tolerance value of each macroinvertebrate taxon (Hilsenhoff, 1988). The index values range from 0–10 with higher values indicating more degraded water quality. The invertebrate community index (ICI) developed by Ohio Environmental Protection Agency (EPA) consists of 10 metrics and has a total possible score of 0–60, with higher scores indicating better water quality (Ohio EPA, 1988). Because ICI scores vary according to catchment size, we adjusted the scoring criteria based on measured stream width. The rapid bioassessment protocol macroinvertebrate index of biotic integrity (RBPIBI) developed by U.S. EPA has eight metrics (Plafkin *et al.*, 1989). The total score ranges from 0 to 48, with higher scores indicating better water quality. The benthic index of biotic integrity (BIBI) developed for the Tennessee River Valley (Kerans and Karr, 1994) has 13 metrics and a total score ranging from 13 to 65, with higher scores indicating better water quality. We excluded the metrics of number of intolerant snail and mussel species and proportion of individuals as *Corbicula* from our calculation because these metrics are species specific and may not be applicable to Wisconsin and Minnesota. The Wisconsin macroinvertebrate index of biotic integrity (WIBI) developed by Weigel (2003) has 9–11 metrics depending upon geographic region, and its score typically ranges from 0 to10, with a higher score indicating better stream quality. We used the 11 metrics for the central-southeastern region on all sites, because this region included the majority of our sampling sites. Limiting analyses to the central-southeastern model reduced confounding variation that would be introduced by using multiple region-specific models. WIBI scores were rescaled between 0 and 10 for easier interpretation. We included all macroinvertebrate taxa in our samples for calculating their assemblage measures, which were typically done by previous studies (e.g., Hilsenhoff, 1988; Ohio EPA, 1988; Kerans and Karr, 1994; Barbour *et al.*, 1997; Weigel, 2003).

2.5. Data analysis

To test the hypothesis that macroinvertebrate communities are substantially different between riffle and snag habitats and to further evaluate the magnitude and nature of the difference, we reported macroinvertebrate taxa found in riffles only, snags only, and those found in both riffles and snags. We also compared HBI and the other 46 macroinvertebrate measures between riffle and snag habitats using a distribution-free sign test (Hollander and Wolfe, 1999) with Bonferroni corrections (Rice, 1989) to evaluate if the difference between the two types of habitats was statistically significant. We used the non-parametric test because about half of the macroinvertebrate measures did not have a normal distribution after various forms of data transformation. We used Bonferroni corrections to eliminate the influence of number of tests on type I error. We also conducted simple linear regressions for the five biotic indices between riffle and snag habitats and visually evaluated the nature of deviation in the index values between riffle and snag habitats along gradients of stream impairment.

To test the hypothesis that macroinvertebrate biotic indices developed for riffles may not be proper for snag habitat, we conducted covariance analysis using multivariate regression (SAS Institute, 1990) to quantitatively test whether regression slopes and intercepts were statistically different between riffle and snag samples in response to human disturbance gradients. Such quantitative differences in regression slopes and intercepts between macroinvertebrate measures from the two habitats were used to evaluate whether the values of the same macroinvertebrate index from the two habitats of the same stream site indicated the same stream health condition.

To further test the hypothesis that macroinvertebrate biotic indices developed for riffles may not be proper for snag habitat, we classified the values of each macroinvertebrate index into five stream health classes (excellent, good, fair, poor, very poor) that have been commonly used in bioassesments (e.g., Hilsenhoff, 1988; Ohio EPA, 1988; Kerans and Karr, 1994; Barbour *et al.*, 1997; Weigel, 2003). The classification was done by dividing the full range values of each index into five equal range groups. Each group was a stream health class. We then compared the difference in classes calculated from riffle and snag data. If snag data misclassified more than 50% of the sites than riffle data did, we considered indices developed for riffles might not be applicable to snag habitat. Such an evaluation provided specific information on how different it would be when applying indices developed from one type habitat to a different type habitat. This comparison made it possible to reject or accept this hypothesis based on practical management rationale.

To test the hypothesis of whether sampling snag habitat alone is sufficient to assess environmental degradation in streams lacking of riffles, we correlated HBI and the other 46 macroinvertebrate measures with catchment land uses. We used Spearman's rank correlation (SAS Institute, 1990) with experimental-wise Bonferroni correction on natural log transformed data to avoid assuming data normal distribution and tests independency, and to improve the data linearity. In conjunction with the aforementioned simple and multivariate regression analyses, we determined whether macroinvertebrate measures from snag habitat were strongly negatively correlated with disturbed land (e.g., urban and agriculture) and positively correlated with undisturbed land (e.g., woodland). If yes, these macroinvertebrate measures from snag habitat alone could be used to assess stream health.

To evaluate whether our results were applicable to streams lacking riffles, we correlated HBI and the other 46 macroinvertebrate measures with instream physicochemical parameters to assess the influence of instream habitat, especially riffles, on macroinvertebrates in riffle and snag habitats using the same correlation procedures. If riffle and riffle-related stream characteristics (e.g., % cobble/gravel and stream gradient) were not significantly correlated with snag macroinvertebrate measures, we interpreted that our results were applicable to streams lacking riffles because our results were not strongly influenced by riffles.

3. Results

3.1. Evaluating the Magnitude and Nature of Differences in Macroinvertebrate Measures between Riffles and Snags

3.1.1. *Difference in Taxa Occurrence*

A diverse macroinvertebrate community, representing 367 taxa, was found (Table I). Many taxa were habitat-specific with 75 taxa found in riffles only and 143 in snags only. In contrast, 149 taxa were found in both riffle and snag habitats.

The majority of the macroinvertebrate taxa occurred in only a few sites (Table I). Among the taxa found in riffles only, 76% of the taxa occurred in four or fewer sites and only 18 taxa occurred in five or more sites. For taxa found in snags only, 78% of the taxa occurred in four or fewer sites and 31 taxa occurred in five or more sites. For taxa found in both riffles and snags, 76% occurred in fewer than 20 sites and 36 taxa occurred in 20 or more sites.

3.1.2. *Differences in Macroinvertebrate Measures*

Sixty-four percent of the macroinvertebrate measures that we tested differed significantly ($p < 0.05$) between riffle and snag habitats (Table II). The largest differences between the two habitats were measures of macroinvertebrate feeding groups (mean percentage difference [MPD] = 143%). Compared to snags, riffles had significantly higher percentages of scraper taxa ($S = 56$, $p < 0.01$) and individuals ($S = 60$, $p < 0.01$), and filter taxa ($S = 46$, $p < 0.01$) and individuals ($S = 48$, $p < 0.01$). However, riffles had significantly lower percentages of predator taxa ($S = 51$, $p < 0.01$) and individuals ($S = 49$, $p < 0.01$), and herbivore taxa ($S = 30$, $p < 0.01$) and individuals ($S = 29$, $p < 0.01$) than snags.

The Ephemeroptera-Plecoptera-Trichoptera (EPT) components were the next most apparent macroinvertebrate measures that differed significantly between riffle and snag habitats (MPD = 32%, Table II). Riffles had more Trichoptera taxa ($S = 29$, $p < 0.01$) and higher percentages of Trichoptera individuals ($S = 33$, $p < 0.01$) than snags. Riffles also had higher percentages of Plecoptera individuals ($S = 29$, $p < 0.01$) and more Ephemeroptera taxa ($S = 21$, $P < 0.05$) than snag habitat.

TABLE I
Macroinvertebrate taxa found in riffle, snag, and riffle and snag habitats

Riffle only			Snag only			Riffle and snag			
No. of taxa and taxa names	No. of sites	Mean individual	No. of taxa and taxa names	No. of sites	Mean individual	No. of taxa and taxa names	No. of sites	Mean individual in riffle	Mean individual in snag
Number of taxa occurred in ≤ 4 sites in riffles only, snags only, and ≤ 20 sites for both riffles and snags									
36 Taxa	1	3	71 Taxa	1	4	45 taxa	1–5	1	2
9 Taxa	2	1	23 Taxa	2	1	38 taxa	6–10	2	2
8 taxa	3	3	14 taxa	3	2	17 taxa	11–15	4	5
4 taxa	4	3	4 taxa	4	2	13 taxa	16–20	3	3
Taxa found in >4 sites in riffles only, snags only, and >20 sites for both riffles and snags									
Coleoptera			**Coleoptera**			**Amphipoda**			
Ectopria leechi sp.	13	2	*Aeshna umbrosa*	9	2	*Hyallela azteca*	36	2	7
Optioservus fastiditus	61	12	*Hydraena* sp.	7	2				
Optioservus trivittatus	6	3	*Hydrochus squamifer*	5	1	**Coleoptera**			
			Liodessus affinis	30	5	*Dubiraphia quadrinotata*	48	1	3
Diptera			*Paracymus subcupreus*	5	1	*Macronychus glabratus*	24	1	3
Atherix variegata	11	3	*Tropisternus glaber*	6	2	*Stenelmis crenata*	41	3	1
Cricotopus trifascia group	11	5	*Tropisternus* sp.	5	2				
Diamesa sp.	10	15				**Diptera**			
Pagastia sp.	8	3	**Diptera**			*Antocha* sp.	31	5	1
			Anopheles sp.	26	7	*Brillia flavifrons* group	29	1	2

(*Continued on next page*)

TABLE I
(*Continued*)

Riffle only			Snag only			Riffle and snag			
No. of taxa and taxa names	No. of sites	Mean individual	No. of taxa and taxa names	No. of sites	Mean individual	No. of taxa and taxa names	No. of sites	Mean individual in riffle	Mean individual in snag
Ephemeroptera			*Brillia flavifrons*	5	1	*Cricotopus bicinctus* group	27	2	2
Baetis tricaudatus	15	6	*Clinotanypus pinguis*	7	2	*Chaetocladius piger* group	32	3	3
Ephemerella inermis sp.	6	2	*Dixa* sp.	8	2	*Chrysops* sp.	21	1	2
Paraleptophlebia mollis	6	22	*Phaenopsectra* sp.	6	1	*Cladotanytarsus vanderwulpi*	22	2	8
Stenonema mediopunctatum	7	11	*Pseudolimnophila* sp.	5	5	*Conchapelopia* sp.	24	2	2
			Symposiocladius lignicola	5	3	*Dicranota* sp.	34	3	1
Megaloptera			*Xlotopus par*	6	1	*Hemerodromia* sp.	43	2	2
Nigronia serricornis	5	3	*Zavrelimyia* sp.	8	5	*Limnophyes* sp.	25	2	2
						Micropsectra sp.	41	3	4
Plecoptera			**Ephemeroptera**			*Microtendipes pedellus* group	39	4	2
Paracapnia angulata	5	8	*Callibaetis* sp.	17	4	*Orthocladius* sp.	33	4	1
			Stenonema femoratum	5	2	*Polypedilum nr. Convictum*	32	3	2
Trichoptera						*Rheotanytarsus* sp.	46	3	3
Ceratopsyche alhedra	7	5	**Heteroptera**			*Simulium vittatum*	38	6	2
Ceratopsyche morosa bifida	6	10	*Aquarius remigis*	8	2	*Stictochironomus* sp.	21	2	2
Cheumatopsyche sp.	49	13	*Belostoma flumineum*	35	2	*Tanytarsus* sp.	29	1	2
Glossosoma sp.	20	8	*Mesovelia mulsanti*	6	1	*Thienemanniella* sp	32	2	3
Psychomyia flavida	11	42	*Microvelia americana*	14	2	*Tipula* sp	53	3	4
			Ranatra fusca	12	3	*Tvetenia* sp	32	5	1

(*Continued on next page*)

TABLE I
(*Continued*)

Riffle only			Snag only			Riffle and snag			
No. of taxa and taxa names	No. of sites	Mean individual	No. of taxa and taxa names	No. of sites	Mean individual	No. of taxa and taxa names	No. of sites	Mean individual in riffle	Mean individual in snag
			Isopoda			**Ephemeroptera**			
			Caecidotea r. racovitzai	9	17	*Baetis brunneicolor*	30	5	12
						Baetis flavistriga	41	6	1
			Odonata			*Caenis* sp.	24	1	3
			Aeshna umbrosa	9	2	*Stenacron interpunctatum*	29	5	2
			Calopteryx aequabilis	20	3				
						Gastropoda			
			Trichoptera			*Physa* sp.	47	5	10
			Limnephilus sp.	9	4				
			Molanna sp.	9	4	**Odonata**			
			Paranyctiophylax sp.	6	1	*Calopteryx maculata*	59	1	5
			Platycentropus sp.	13	3				
			Ptilostomis sp.	7	2	**Trichoptera**			
						Ceratopsyche bronta	25	6	6
						Ceratopsyche slossonae	42	10	1
						Chimarra aterrima	25	7	6
						Hydropsyche betteni	71	9	4
						Pycnopsyche sp.	38	1	2

Taxon names were listed if they occurred in greater than four sites for riffles only and snags only, or occurred in greater than 20 sites for riffles and snags. The numbers of taxa found in less than or equal to four riffle sites, snag sites, and in greater than or equal to 20 sites for both riffles and snags were list in the first four rows.

TABLE II
Comparison of macroinvertebrate measures between riffles and snags for mean, standard error (SE), and distribution-free sign test statistic (*S*) and *P* value

	Riffle		Snag		Sign Test	
Macroinvertebrate measure	Mean (1SE)	Range	Mean (1SE)	Range	*S*	*P*
Feeding morphology and trophic group						
Filterer abundance (%)[1]	27.1 (1.5)	0.0–82.6	11.3 (1.2)	0.0–70.0	48	<0.01
Filterer taxa (%)	23.7 (0.8)	0.0–52.2	14.4 (0.8)	0.0–45.5	46	<0.01
Gatherer abundance (%)	40.6 (2.0)	2.3–98.6	58.4 (2.1)	9.0–98.9	41	<0.01
Gatherer taxa (%)[4]	35.8 (0.8)	8.7–66.7	32.5 (1.0)	8.3–87.5	23	<0.01
Herbivore abundance (%)	3.5 (0.5)	0.0–37.5	8.7 (0.9)	0.0–51.1	29	<0.01
Herbivore taxa (%)	6.6 (0.4)	0.0–20.0	11.2 (0.6)	0.0–25.7	30	<0.01
Omnivore abundance (%)[1]	2.4 (0.4)	0.0–37.7	3.4 (0.7)	0.0–59.5	12	>0.05
Predator abundance (%)[1]	2.7 (0.3)	0.0–24.1	9.3 (0.8)	0.0–51.2	49	<0.01
Predator taxa (%)	8.2 (0.6)	0.0–40.0	18.6 (0.8)	0.0–46.2	51	<0.01
Scraper abundance (%)[1,4]	22.5 (1.4)	0.0–85.4	4.6 (0.6)	0.0–39.4	60	<0.01
Scraper taxa (%)	16.2 (0.7)	0.0–40.0	8.4 (0.6)	0.0–41.7	56	<0.01
Scraper/filter (No., ratio)[3]	99.4 (14.6)	0.0–1417.0	72.2 (17.5)	0.0–1742.9	40	<0.01
Shredder abundance (%)[3]	0.1 (0.0)	0.0–3.9	0.4 (0.1)	0.0–12.7	9	>0.05
Shredder taxa (%)	0.4 (0.1)	0.0–7.4	1.2 (0.2)	0.0–12.9	9	>0.05
Taxa richness and composition						
Abundance (No./sample)[1]	229.9 (12.8)	59–1585	202.5 (16.9)	20–2257	15	>0.05
Chironomid abundance (%)	10.7 (1.1)	0.0–65.3	11.6 (1.3)	0.0–86.7	7	>0.05
Chironomid taxa (%)[4]	23.9 (1.2)	0.0–68.8	22.5 (1.2)	0.0–64.0	10	>0.05
Community similarity index[3]	8.5 (0.3)	3.7–26.9	8.6 (0.5)	3.3–32.8	17	>0.05
Diptera abundance (%)	4.8 (0.3)	0.0–14.7	5.4 (0.3)	0.0–18.2	8	>0.05
Diptera taxa (%)[4]	35.2 (1.2)	0.0–73.3	31.6 (1.2)	0.0–80.0	35	<0.01
Diptera (No. of taxa)[2]	9.5 (0.4)	0–28	9.0 (0.5)	0–24	15	>0.05
Ephemeroptera abundance (%)[2]	14.1 (0.3)	0.0–53.9	15.9 (1.5)	0.0–77.1	2	>0.05
Ephemeroptera (No. of taxa.)[1,2]	3.6 (0.2)	0–9	2.9 (0.2)	0–8	21	<0.05
EPT abundance (%)	40.8 (1.9)	0.0–90.1	28.7 (1.9)	0.0–85.3	23	<0.01
EPT abundance (No./sample)[4]	8.3 (0.3)	1.019.1	6.0 (0.3)	1.0–19.4	27	<0.01
EPT taxa (%)[4]	35.4 (1.2)	0.0–72.4	26.0 (1.1)	0.0–62.5	40	<0.01
EPT (No. of taxa)[2,3]	9.5 (0.4)	0–22	7.0 (0)	0–24	39	<0.01
EPT/Chironomid (No., ratio)[3]	58.7 (5.0)	0.0–274.7	61.1 (0.0)	0.0–696.1	9	>0.05
Isopoda abundance (%)	8.8 (1.4)	0.0–97.2	15.2 (0.0)	0.0–95.2	25	<0.01
Isopoda taxa (%)[4]	3.4 (0.3)	0.0–20.0	4.2 (0.0)	0.0–28.6	3	>0.05
Isopoda (No. of taxa)[4]	1.2 (0.0)	1.0–1.7	1.3 (1.0)	1.0–1.7	36	<0.01
Oligochaeta abundance (%)[1]	2.2 (0.6)	0.0–60.0	1.5 (0.0)	0.0–34.1	10	>0.05
Plecoptera (No. of taxa)[1]	0.5 (0.1)	0–6	0.3 (0)	0–5	7	>0.05
Plecoptera abundance (%)	1.6 (0.4)	0.0–30.2	0.7 (0.0)	0.0–13.9	29	<0.01
Shannon-Wiener diversity index	2.4 (0.1)	0.2–3.3	2.1 (0.3)	0.3–3.5	27	<0.01
The most abundant taxon (No. %)[3]	30.7 (1.3)	11.1–97.2	41.2 (9.2)	9.2–95.2	23	<0.01
The other Dipteran and non-insect abundance (%)[2]	38.6 (2.6)	2.6–100.0	54.5 (2.1)	1.9–99.4	25	<0.01
Total number of taxa[1,2,3,4]	26.6 (0.8)	8–52	27.1 (1.0)	6.0–53.0	7	>0.05
Tribe Tanytarsini midge (No., %)[2]	1.7 (0.3)	0.0–38.0	2.7 (0.5)	0.0–46.2	2	>0.05

(*Continued on next page*)

TABLE II
(*Continued*)

	Riffle		Snag		Sign Test	
Macroinvertebrate measure	Mean (1SE)	Range	Mean (1SE)	Range	*S*	*P*
Trichoptera abundance (%)[2]	25.1 (12.8)	0.0–72.5	12.1 (1.1)	0.0–61.3	33	<0.01
Trichoptera (No. of taxa)[2]	5.4 (3.8)	0–13	3.8 (0.2)	0–14	29	<0.01
Two most abundant taxa (No., %)[2]	45.9 (1.4)	21.1–98.0	55.6 (1.7)	17.8–98.1	27	<0.01
Tolerance						
Depositional abundance (%)	47.7 (1.7)	1.7–98.3	56.1 (2.1)	3.2–99.5	21	<0.05
Depositional taxa (%)[4]	36.5 (0.8)	12.5–60.0	42.5 (1.0)	11.8–87.5	30	<0.01
Hilsenhoff biotic index[3]	4.7 (0.1)	0.8–8.0	5.4 (0.1)	2.5–8.0	40	<0.01
Mean tolerant value[4]	4.9 (0.1)	1.9–8.5	5.3 (0.1)	2.7–9.0	41	<0.01
Tolerant organism abundance (%)[2]	2.7 (0.7)	0.0–66.0	2.1 (0.5)	0.0–51.2	8	>0.05

Note. EPT represents members of Ephemeroptera, Plecoptera, and Trichoptera orders. The significant levels of *P*-value was adjusted using Bonferroni correction.
[1]Metrics of B-IBI (Kerans and Karr 1994).
[2]metrics of ICI (Ohio EPA 1987).
[3]metrics of RBPIBI (Plafkin *et al.* 1989).
[4]metrics of WIBI (Weigel 2003).

3.2. Evaluating if Macroinvertebrate Indices Developed for Riffles Could be Used for Snags

The intercepts, slopes, and coefficients of determination of regressions between urban and agricultural land uses versus macroinvertebrate biotic indices differed markedly between riffles and snags (Table III). The regression intercepts between land uses and HBI, BIBI, and ICI were significantly higher, whereas intercepts between land uses and WIBI were lower for riffle than for snag samples. The regression intercepts between urban land use and RBPIBI were also significantly higher for riffle than for snag samples. Regression slopes between agricultural land use and BIBI and ICI were the only regression pairs that showed significant difference between riffle and snag habitats.

The index values calculated from riffles grouped 43 to 66% of the study sites in the same stream health classes as that from snags (Figure 2). The classes assigned by HBI were the most similar between riffle and snag samples, with 66% of the sites in the same class and 34% sites differing by one class. The classes assigned by the other four indices were similar, with 41–50% sites being in the same class, 37–51% sites being one class different, 6–18% sites being two classes different, and 1–2% sites being three classes different.

The values of biotic indices calculated from snags did not predict the values calculated from riffle very well (Figure 3). Among the five indices, HBI was the highest, in which values for snag samples explained about 70% of the variance in riffle samples. For the other four indices, index values calculated from snags explained less than 50% of the variance in scores calculated from riffle samples.

TABLE III

Correlation coefficients (r), intercepts, and slopes of simple linear regressions between macroinvertebrate biotic indices and urban or agricultural land uses

		Urban					Agriculture				
		Riffle	Snag	F	WL	P	Riffle	Snag	F	WL	P
HBI	r	0.42***	0.33***			0.51***	0.32***				
	Intercept	5.5	4.9	76.6	0.64	<0.01	6.6	5.6	35.3	0.79	<0.01
	Slope	0.03	0.02	1.4	0.99	0.23	0.02	0.01	1.9	0.99	0.17
WIBI	r	0.26***	0.23***			0.24**	0.19				
	Intercept	5.9	6.5	19.4	0.87	<0.01	6.5	7.0	3.6	0.97	0.06
	Slope	0.02	0.01	0.1	1.00	0.94	0.02	0.01	0.6	1.00	0.42
BIBI	r	0.57***	0.46***			0.30***	0.45***				
	Intercept	39.5	37.0	29.6	0.82	<0.01	42.1	40.8	3.8	0.97	0.05
	Slope	0.15	0.12	2.6	0.98	0.11	0.05	0.08	4.1	0.95	0.04
ICI	r	0.46***	0.35***			0.49***	0.47***				
	Intercept	35.4	30.4	49.5	0.73	<0.01	42.2	39.9	7.1	0.94	<0.01
	Slope	0.22	0.26	1.9	0.99	0.25	0.14	0.19	4.2	0.96	0.04
RBPIBI	r	0.46***	0.39***			0.50***	0.44***				
	Intercept	21.6	20.0	7.3	0.94	<0.01	28.5	26.6	0.9	0.99	0.45
	Slope	0.19	0.17	0.6	1.00	0.46	0.14	0.13	0.2	1.00	0.63

Streams with greater than 10% urban in watershed were excluded when macroinvertebrate measures were regressed with agricultural land use. The differences in regression slopes and intercepts between riffle and snag samples were tested by analysis of covariance using multivariate regression. WL = Wilks' Lambda values. ***indicates regression r with $p < 0.01$ and **indicates r with $p < 0.05$. See Table II for biotic index abbreviations.

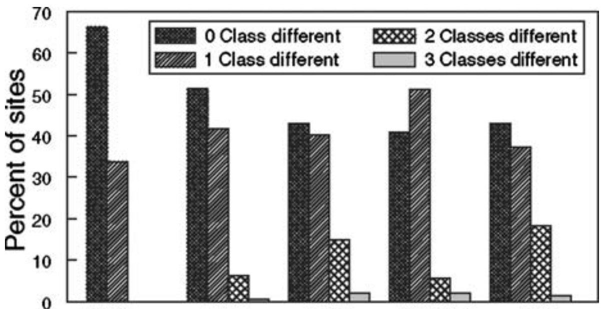

Figure 2. Percent difference between riffle and snag samples in stream sites that were classified into different health groups. The classification was done by dividing the full range of each index values for both riffles and snags into five health groups: excellent, good, fair, poor, and very poor. Although the classes used here is relative between riffle and snag habitats, the plots indicate that misclassifying stream into a different class resulted from habitat-specific sampling can lead to substantial biased conclusion during environmental impairment assessment.

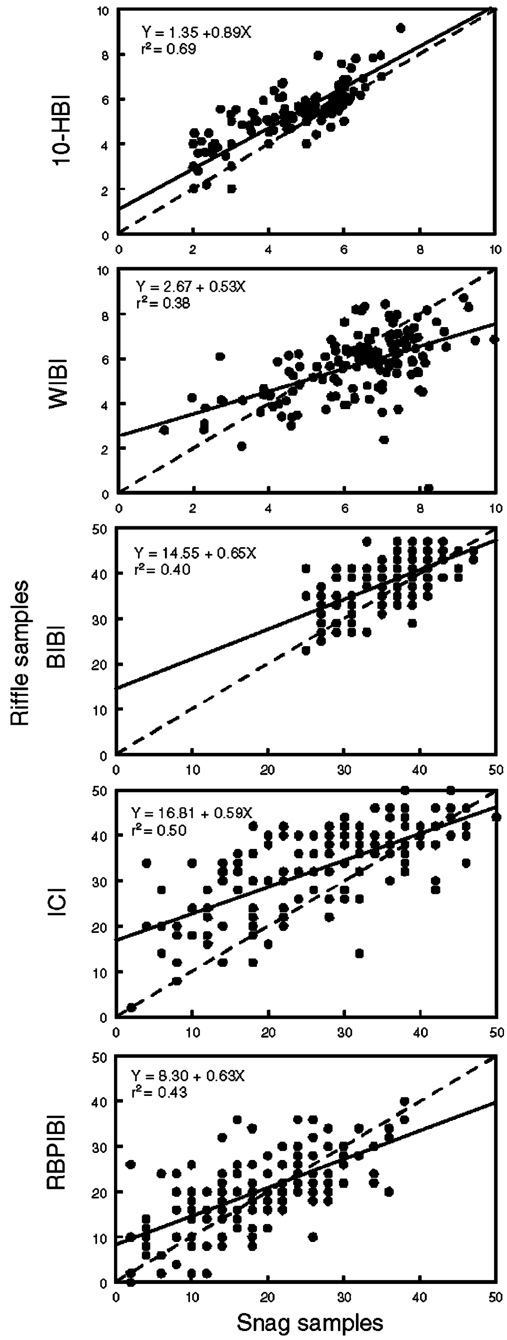

Figure 3. Plots between macroinvertebrate biotic index values calculated from snag and riffle samples. Broken lines indicate that the riffle and snag index values are equal. See Table II for biotic index abbreviations.

The values of WIBI and RBPIBI calculated from snag samples underestimated environmental impairment relative to that calculated from riffle samples when streams had low index values, and overestimated environmental impairment relative to riffle when streams had high index values (Figure 3). The values of ICI calculated from snag samples underestimated environmental impairment relative to that calculated from riffle samples when streams had low index values. For streams having least impacted conditions, the values of ICI calculated from snag and riffle habitat were similar.

3.3. Evaluating sampling snag alone could be sufficient for assessing stream health

Many of the snag macroinvertebrate measures significantly correlated with disturbed lands and woodland (Figure 4, Appendix 1), implying that sampling snag habitat alone should be sufficient for assessing stream health. About 57% of the 47 snag macroinvertebrate measures correlated with urban, 23% correlated with agricultural, and 53% correlated with woodland. Less than 13% snag macroinvertebrate measures correlated with grassland, wetland, and catchment size. Similarly, 53% of the 47 riffle macroinvertebrate measures significantly correlated with urban, 23% correlated with agricultural, and 60% correlated with woodland. Less than 17% riffle macroinvertebrate measures correlated with grassland, wetland, and catchment size. No snag or riffle macroinvertebrate measures correlated with open water.

Although the numbers of macroinvertebrate measures correlated with catchment land uses for snags and riffles were similar, the specific macroinvertebrate variables correlated with land uses were substantially different. Among the 33 significant correlations between urban land use and macroinvertebrate measures, 19 correlated with both riffle and snag samples, six with riffle samples only, and seven with snag samples only. Among the 17 significant correlations between agricultural land use and macroinvertebrate measures, five correlated with both habitat samples, six with riffle samples only, and six with snag samples only. For the 32 correlations with woodland, 20 correlated with both habitat samples, eight with riffle samples only, and five with snag samples only.

Less than 17% of the macroinvertebrate measures correlated with grassland, wetland, open water, or catchment area, and the macroinvertebrates from riffle and snag habitats correlated with these catchment characteristics differently (Figure 4, Appendix 1). Among the six macroinvertebrate measures correlated with grassland, one was riffle and five were snag samples. Among the 14 macroinvertebrate measures correlated with wetland, three correlated with both riffle and snag samples, five with riffle samples only, and three with snag samples only. For those correlated with catchment area, only one macroinvertebrate measure was correlated with both riffle and snag, none with riffle, and four with snag samples only.

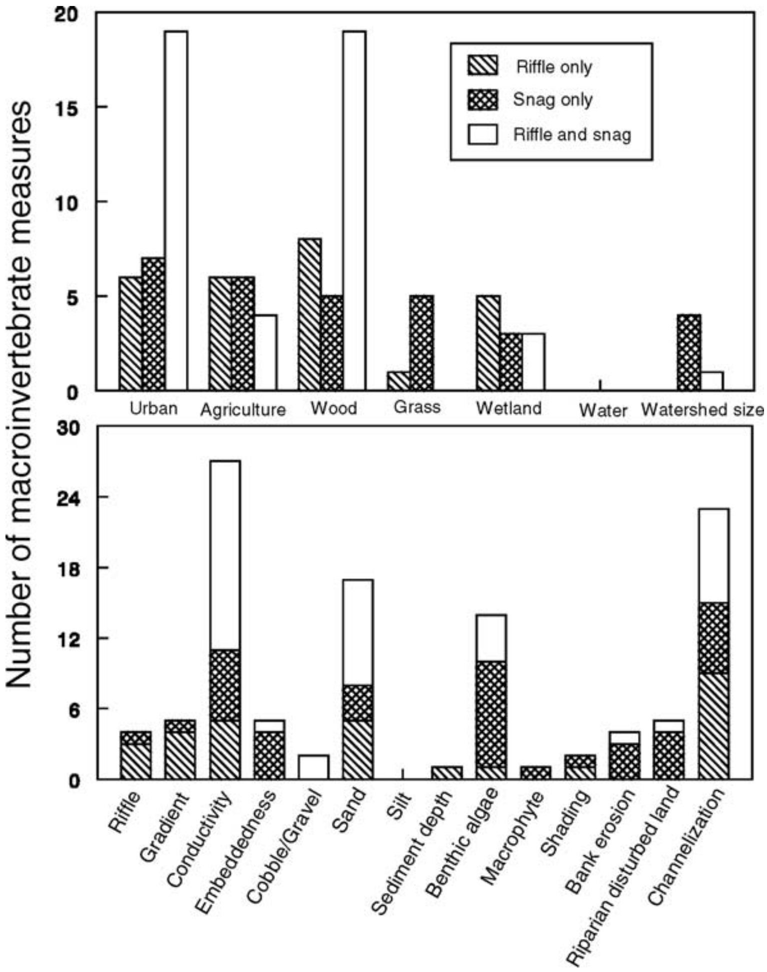

Figure 4. Number of macroinvertebrate measures significantly ($p < 0.05$) correlated with watershed land uses (%), watershed size (km^2), and reach habitats. All habitat variables were in percent except gradient (m/km), conductivity (μm/s), and sediment depth (cm). See appendixes 1 and 2 for variable abbreviations.

3.4. Evaluating whether our results could be applied to streams without riffles

Very few snag macroinvertebrate measures were correlated with percent riffle or riffle-associated habitats (Figure 4, Appendix 2). Among the 47 snag macroinvertebrate measures, only one measure significantly correlated with percentage of riffle, one correlated with stream gradient, and two correlated with cobble/gravel substrates. Similarly, among the 47 riffle macroinvertebrate measures, only three measures were significantly correlated with percentage of riffle, five correlated with gradient, and two correlated with cobble/gravel substrates. Among the four significant correlations between percentage of riffle and macroinvertebrate measures and among the six significant correlations between gradient and macroinvertebrate measures, none were correlated with both riffle and snag samples. For the four correlations with cobble/gravel, all were correlated with both riffle and snag habitats.

The predominant instream habitats correlated with macroinvertebrate measures were conductivity, sand substrate, benthic algae, and channelization (Figure 4, Appendix 2). Water conductivity and sand substrate correlated predominantly with both riffle and snag samples; benthic algae correlated predominantly with snag samples; and channelization correlated about equally with riffle, snag, and both riffle and snag samples.

In contrast, substrate embeddedness, bank erosion, and riparian disturbance were predominantly correlated with snag or both riffle and snag samples. Among the five macroinvertebrate measures correlated with embeddedness, four were snag samples and one was derived from both snag and riffle samples; among the four correlated with bank erosion, three were snag samples and one was from both riffle and snag samples; and among the five correlated with riparian disturbance, four were snag samples and one was from both snag and riffle samples.

4. Discussion

Our results are in concordance with our hypothesis that benthic macroinvertebrate measures from either riffle or snag habitat alone can indicate human disturbance on stream ecosystems. However, all three types of macroinvertebrate measurements that we tested, including taxa occurrence, tolerance and functional feeding groups of taxa and individuals, and the biotic indices showed significant difference between riffle and snag habitats. If macroinvertebrate samples obtained from riffles or multihabitat including riffles in the reference streams were compared with samples from snags only in the test streams or if a biotic index developed from one type habitat is applied to data collected from different habitat types, this difference could potentially bias the results of the environmental assessment.

4.1. Influence of differences in macroinvertebrate measures between riffles and snags

We found a considerable number of taxa that were unique to either riffles or snags, which potentially influences the outcome of macroinvertebrate environmental assessments that rely on taxon richness or taxon presence/absence. Most macroinvertebrate indices of biotic integrity (e.g., Ohio EPA, 1988; Kerans and Karr, 1994; Barbour *et al.*, 1997; Weigel, 2003) include total taxa richness as a key component of their metrics. Eleven of the 17 taxon measures that we summarized are included in at least one of the four multimetric indices that we cited. There is an apparent need to take into account the influence of habitat-specific sampling on taxon occurrence for macroinvertebrate indices that use data from multiple habitats or from the most-productive habitat if the compositions of the habitat from which macroinvertebrates were collected are different between reference and test sites.

The significant difference in macroinvertebrate measures between riffle and snag habitats, especially measures of feeding function and ETP groups, also potentially influences the outcome of macroinvertebrate environment assessments using multimetric indices. Measures of feeding and EPT groups have been included in virtually all macroinvertebrate indices of biotic integrity, including the RBPIBI (Plafkin *et al.*, 1989), BIBI (Kerans and Karr, 1994), ICI (Ohio EPA, 1988), and WIBI (Weigel, 2003). It is substantial that for about half of our macroinvertebrate feeding measures, values for one habitat were more than double those for the other habitat, and for the other half of feeding measures, values for one habitat were 50% higher on average than for the other habitat. The EPT measures were 32% higher for one habitat than for the other habitat. If not corrected, such a large difference in the two macroinvertebrate groups between riffle and snag habitats could introduce substantial bias into the bioassessment results.

Our finding that a considerable number of taxa occurred in less than 5% of the sites, and that the number of these rare taxa were not correlated with agricultural or urban land uses ($r < 0.15$, $p > 0.2$) could also potentially affect bioassessments that rely on taxa occurrence or multiple metrics. Excluding rare taxa in the index calculation as the Australian version of the predictive model did (e.g., Parsons and Norris, 1996; Marchant *et al.*, 1997) could potentially minimize such an effect.

Other studies also found that habitat-specific sampling affects measures of macroinvertebrate functional feeding groups. In the recognition of specific-habitat sampling effects, the Rapid Bioassessment Protocol III (Plafkin *et al.*, 1989) recommended sampling riffle/run benthic communities to measure scrapers and filtering collectors, and sampling coarse particulate organic mater to provide an additional measure of effects of shredders. In studying 54 reference streams and 10 streams with some physical habitat alteration in Missouri, Rabeni (2000) also reported that macroinvertebrate communities collected from the same habitat type at different streams were more similar than those collected from different habitat types at the same stream. Because sampling habitats have substantial influence on the occurrence and abundance of macroinvertebrate feeding functional and EPT groups, caution needs to be taken when comparisons are made between streams with data collected from habitats that are partially or entirely different.

4.2. Influence of Differences in Biotic Indices between Riffles and Snags

Our three approaches used to evaluate the difference in multimetric indices between riffles and snags provided both quantitative and qualitative assessments of the nature and magnitude of biases introduced by applying indices calibrated for riffle to data from snags for bioassessment. First, the significantly higher regression intercepts between urban land use and HBI, BIBI, ICI, and RBPIBI, and between agricultural land use and HBI and ICI for riffle than for snag statistically quantified such biases in terms of index scores. Second, the comparison of bioassessment classes calculated based on multimetric index scores for riffles and snags assessed the bias in terms of biological and management meanings. Finally, the visual evaluation of the plots of the index scores between riffles and snags provided insights on how such a difference changed following a degradation gradient. Although we could not find similar studies that quantified the difference in multimetric indices between riffle and snag habitats, Hooper (1993) and Shepard (2002) reported that stream health classifications evaluated using HBI indicated poorer conditions based on snag versus riffle samples for the same high quality streams in Wisconsin, which is consistent with our conclusion.

Macroinvertebrate multimetric indices are a major bioassessment tool that is broadly used in North America and elsewhere. Because a multimetric index is influenced by regional physicochemical and biological settings, many versions have been developed for different geographic regions (e.g., Ohio EPA, 1988; Plafkin *et al.*, 1989; Kerans and Karr, 1994; Weigel, 2003). The seriousness of impairment at test sites is assessed by comparing conditions of the reference sites within a relatively homogeneous region, such as ecoregions (Omernik and Gallant, 1988). This approach with additional quantifiers, such as stream size, elevation, and riparian vegetation, has potentially minimized the influence of regional variation in climate, geology, soil, landscape topography, land cover, and thermal and hydrological regimes. However, it does not take into account the influence of variation in instream habitats from which the macroinvertebrates were collected. For example, the Northern Lakes and Forest Ecoregion that encompasses northern Michigan, Wisconsin, and Minnesota has stream gradients from nearly flat (less than $0.3\,\mathrm{m\,km^{-1}}$) to steep ($36\,\mathrm{m\,km^{-1}}$) and about half of the sites contain well-developed riffles in addition to pools and runs, but the remainder have only pools and runs (Wang *et al.*, 2003).

Although the sampling habitat types vary among regions, the major sampling protocols for natural habitat are proportional multiple habitats and the most-productive habitat. The multi-habitat protocol attempts to sample all available individual habitats approximately proportional to their occurrence, such as the Rapid Bioassessment Protocols (Barbour *et al.*, 1997) and the predictive model (Wright *et al.*, 1984). The most-productive habitat protocol samples riffle habitat when available and samples other rocky or snag habitats when riffles are not available (e.g., Moulton *et al.* 2002). Obviously, the influences of habitat-specific sampling depend on the difference in the proportions of each habitat type between reference and test sites. The larger the difference is in the proportions of habitat types between reference and test sites, the bigger the bias introduced in the output of the bioassessment; the largest bias would be from comparing sites with and without riffles. Therefore, even if the multi-habitat or the most-productive habitat sampling approach is applied to a relatively homogenous ecoregion, the bioassessment results could differ between stream sites with well-developed rocky substrate and those with only a silt or sand bottom even if their stream health conditions are the same (Weigel *et al.*, 2003).

4.3. Effects of habitat-specific sampling on ability to assess impairments

The ultimate goal of benthic macroinvertebrate assessment is to evaluate whether a stream is impaired by human activities so that corresponding actions can be taken to prevent further degradation or to restore stream health. Bioassessment indices should provide sufficient sensitivity and precision to ensure that stream health can be evaluated with confidence. Our results imply that applying indices developed for riffle to snag samples or mixing riffle and snag samples could overestimate or underestimate stream health conditions, depending on the indices used and the stream health conditions. Such biases lower our confidence in bioassessment.

One way to minimize the influence of habitat-specific sampling is to classify reference and test sites within a relatively homogenous region based on factors that determine the types and abundance of instream habitat from which macroinvertebrate data are collected. The goal of this classification is to identify a test site as a member of the population of reference sites. In the current bioassessment literature, most stream classifications have largely focused on identifying relatively homogenous bioregions that determine reference conditions for a sizeable population of water bodies to enable broad-scale bioassessment (e.g., Hughes, 1995; Weigel, 2003). Such a classification is necessary for conducting broad geographical bioassessment. Further classification of tream sites within a bioregion based on stream size, substrate size, gradient, riparian conditions, and thermal and hydrological regimes would minimize or largely eliminate the influence of habitat-specific sampling.

Developing multimetric indices for a specific habitat type, such as snag for low gradient streams, may improve our ability of bioassessment. Biotic indices calculated from both riffle and snag habitats significantly correlated with urban and agricultural land uses in our study implying that both riffle and snag samples can independently detect human disturbance using macroinvertebrate communities. The substantial difference in stream health groups classified by riffle and snag samples and the significant difference in regression intercepts and slopes between riffle and snag samples indicate that macroinvertebrate measures for each habitat has its own sensitivity and correlation scales relative to environmental conditions. Wang and Kanehl (2003) also found that percent scraper abundance was more sensitive for riffles than for snags. The percent scraper abundance for riffles decreased from 45% to 8% as urban imperviousness increased from 8 to 15%, while the percent scraper abundance for snags never exceeded 5% for the same streams regardless of the levels of imperviousness. Hence, having separate macroinvertebrate indices that calibrate specifically for riffles or snags may substantially improve our ability to detect stream degradation.

Previous studies also reported that macroinvertebrate indices developed from different habitat samples can adequately assess environmental condition. Kerans and Karr (1994) showed that BIBI developed from pool and riffle data could correctly rank sampling sites independently in the Tennessee River Valley, but the rankings were not always consistent between pool and riffle samples. Parsons and Norris (1996) evaluated the effect of riffle, edge, pool-rock, and macrophyte habitat data on the outcome of bioassessment in Australia. They found that macroinvertebrate collections from the same habitat at different sites were more similar than collections from different habitats within a site for reference streams. However, when comparing the observed/expected taxon ratio for the test sites, all of the predictive models developed separately for each habitat type were effective in detecting biological impairment.

To determine whether our results are transferable to streams without riffles is difficult because no two streams are exactly the same under natural conditions. Such conditions can only be obtained under controlled experiment. Our approach compared data between riffles and snags from the same sampling site, in which the two data sets were from exactly the same stream condition. We hypothesized that if the amount of riffles or riffle-related site gradient or coarse substrates did not correlate with the macroinvertebrate measures for snags from sites having riffles, then our results could be transferred to streams without riffle. As our results showed, very few snag macroinvertebrate measures correlated with percent riffle and riffle-associated habitats. Among the 47 snag macroinvertebrate measures we tested, only percent Isopoda taxa correlated with riffle, percent predator taxa correlated with gradient, and percentages of Diptera and scraper taxa correlated with cobble/gravel substrates. Such a weak influence of riffle and riffle-related habitats on our measures implies that our results could be transferable to streams without riffles.

In summary, our results showed that macroinvertebrate taxon occurrence, assemblage measures, and biotic indices were significantly different between riffle and snag habitats, although data from both habitats responded equally well to human disturbance. Because 64% of the tested macroinvertebrate measures were significantly different between the two habitats, and index values from one habitat type explained less than 50% of the variance for the other habitat, we concluded that samples from riffles and snags should not be mixed and the index developed for one habitat type may not be applied to data collected from other habitat types. We recommend sampling snag habitat in streams that naturally lack riffles and develop an index of biotic integrity specifically using snag samples. At present, streams without riffles or other rocky substrates are the least studied partly because of the lack of suitable sampling and evaluation tools (Humphries *et al.*, 1998). Further evaluation of the feasibility of developing a snag-sampling protocol and a biotic index will improve our ability to assess stream impairment for low gradient streams.

APPENDIX I

Watershed size or land-use variables that significantly correlated (r, $p < 0.05$, Spearman's rank correlation) with macroinvertebrate measures for riffles (R) and snags (S). Numbers in superscript indicate metrics used in different indices of biotic integrity (see Table II for explanation). EPT represents members of Ephemeroptera, Plecoptera, and Trichoptera

	Urban (%)		Agriculture (%)		Wood (%)		Grass (%)		Wetland (%)		Water (%)		Watershed (km²)	
Macroinvertebrate measure	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
Feeding morphology														
Filterer abundance (%)[1]	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–
Filterer taxa (%)	0.3	–	–	–	–	–	–	0.3	–	–	–	–	–	–
Gatherer abundance (%)	0.4	0.3	–	–	–	–	–	–	–	–	–	–	–	–
Gatherer taxa (%)[4]	–	0.3	–	–	0.3	–	–	–	0.3	–	–	–	–	–
Herbivore abundance (%)	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–
Herbivore taxa (%)	–	0.4	–	–	0.3	0.5	–	–	–	–	–	–	–	–
Omnivore abundance (%)[1]	–	–	0.4	0.3	–	–	–	–	–	–	–	–	–	0.3
Predator abundance (%)[1]	–	–	0.4	–	0.4	–	–	–	–	–	–	–	–	–
Predator taxa (%)	0.3	–	0.5	0.2	0.3	–	–	–	–	–	–	–	–	–
Scraper abundance (%)[1,4]	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–
Scraper taxa (%)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Scraper/filter (number, ratio)[3]	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–
Shredder abundance (%)[3]	–	–	–	–	–	–	–	–	–	–	–	–	0.4	0.4
Shredder taxa (%)	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3
Taxa richness and composition														
Abundance (number/sample)[1]	–	0.4	–	–	0.3	–	–	–	–	–	–	–	–	–
Chironomid abundance (%)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chironomid taxa (%)[4]	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Community similarity index[3]	0.5	0.4	–	–	0.5	–	–	–	0.3	–	–	–	–	–
Diptera abundant (%)	–	0.3	–	–	–	0.3	–	–	–	–	–	–	–	–
Diptera taxa (%)[4]	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–
Diptera (number of taxa)[2]	0.5	–	–	0.3	0.4	0.3	–	–	0.3	–	–	–	–	–

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Macroinvertebrate measure	Urban (%)		Agriculture (%)		Wood (%)		Grass (%)		Wetland (%)		Water (%)		Watershed (km^2)	
	R	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
Ephemeroptera abundance (%)[2]	0.3	0.3	–	–	0.3	0.4	–	–	–	–	–	–	–	–
Ephemeroptera (number of taxa)[1,2]	0.5	0.4	–	–	0.4	0.3	–	–	–	–	–	–	–	–
EPT abundance (%)	0.4	0.4	–	0.3	0.4	0.5	–	–	–	–	–	–	–	–
EPT abundance (number/sample)[4]	0.4	0.3	–	–	0.4	0.5	–	–	–	–	–	–	–	–
EPT taxa (%)[4]	0.5	0.4	–	–	0.5	0.4	–	–	0.3	–	–	–	–	–
EPT (number of taxa)[2,3]	0.5	0.4	–	0.3	0.5	0.6	–	–	0.3	0.3	–	–	–	–
EPT/Chironomid (number, ratio)[3]	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–
Isopoda abundance (%)	0.3	0.3	–	–	0.3	0.3	–	–	–	–	–	–	–	–
Isopoda taxa (%)[4]	0.5	0.6	–	–	0.5	0.4	–	–	–	–	–	–	–	–
Isopoda (number of taxa)[4]	0.3	0.3	0.3	–	0.7	0.5	–	–	–	–	–	–	–	–
Oligochaeta abundance (%)[1]	0.5	–	–	–	–	–	–	–	–	–	–	–	–	–
Plecoptera abundance (%)	–	0.3	0.3	0.4	0.4	0.4	–	–	–	–	–	–	–	–
Plecoptera (number of taxa)[1]	–	–	0.4	0..3	0.5	0.4	0.3	–	–	–	–	–	–	–
Shannon-Wiener diversity index	0.5	0.4	–	–	0.3	0.3	–	–	–	0.3	–	–	–	–
The most abundant taxon (%)[3]	0.4	0.4	–	–	0.3	–	–	–	–	–	–	–	–	–
The other Diptera and non-insect abundance (%)[2]	0.5	0.5	–	–	0.4	0.4	–	–	0.3	0.3	–	–	–	0.3
Total number of taxa[1,2,3,4]	0.4	0.3	–	–	0.3	0.3	–	–	–	0.3	–	–	–	–
Tribe Tanytarsini midge (No., %)[2]	–	–	0.3	–	–	0.3	–	–	–	–	–	–	–	–
Trichoptera abundance (%)[2]	–	0.3	–	0.3	–	0.3	–	0.3	–	–	–	–	–	0.2
Trichoptera (number of taxa)[1,2]	0.5	0.4	0.2	0.3	0.5	0.5	–	0.2	0.3	0.3	–	–	–	–
Two most abundant taxa (No. %)[1]	0.5	0.3	–	–	0.3	–	–	–	0.3	–	–	–	–	–
Tolerance														
Depositional abundance (%)	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–
Depositional taxa (%)[4]	–	0.4	–	0.3	0.3	0.5	–	0.2	–	0.3	–	–	–	–
Hilsenhoff biotic index[3]	0.5	0.4	0.3	–	0.6	0.4	–	–	–	–	–	–	–	–
Mean tolerant value[4]	0.5	0.5	0.3	–	0.7	0.5	–	–	–	–	–	–	–	–
Tolerant organism (No. %)[2]	0.5	–	0.2	–	–	–	–	–	–	–	–	–	–	–

APPENDIX II

Selected instream habitat variables that were significantly correlated (r, $p < 0.05$, Spearman's rank correlation) with macroinvertebrate measures for riffles (R) and snags (S)

	Riffle (%)		Grad-ient (m/km)		Condu-ctivity (um/s)		Embed-dedness (%)		Cobble/ gravel (%)		Sand (%)		Silt (%)		Sediment depth (cm)		Benthic algae (%)		Macro-phyte (%)		Shad-ing (%)		Bank erosion (%)		Disturbed land (%)		Chann-elization (%)	
Macroinvertebrate measure	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
Feeding and morphology																												
Filterer abundance (%)[1]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–
Filterer taxa (%)	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	–
Gatherer abundance (%)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	0.3	–	–
Gatherer taxa (%)[4]	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Herbivore abundance (%)	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3
Herbivore taxa (%)	–	–	–	–	0.3	0.5	–	–	–	–	0.3	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.4
Omnivore abundance (%)[1]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3
Predator abundance (%)[1]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–
Predator taxa (%)	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Scraper abundance (%)[1,4]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–
Scraper taxa (%)	–	–	–	–	–	–	–	0.3	0.3	0.3	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Scraper/filter (No., ratio)[3]	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.4
Shredder abundance (%)[3]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Shredder taxa (%)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Taxa richness and composition																												
Abundance (No./sample)[1]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3
Chironomid abundance (%)	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chironomid taxa (%)[4]	–	–	0.3	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Community similarity index[3]	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	–
Diptera abundant (%)	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Diptera taxa (%)[4]	0.3	–	0.3	–	–	–	0.3	0.3	0.3	0.3	0.3	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Diptera (No. of taxa)[2]	0.3	–	–	–	–	–	–	0.3	–	–	0.3	0.3	–	–	–	–	–	–	–	–	0.3	–	–	–	–	0.3	–	–
Ephemeroptera abundance (%)[2]	–	–	–	–	0.3	0.3	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–
Ephemeroptera (No. of taxa)[1,2]	–	–	–	–	0.4	0.4	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–

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Macroinvertebrate measure	Riffle (%)		Grad-ient (m/km)		Condu-ctivity (um/s)		Embed-dedness (%)		Cobble/gravel (%)		Sand (%)		Silt (%)		Sediment depth (cm)		Benthic algae (%)		Macro-phyte (%)		Shad-ing (%)		Bank erosion (%)		Disturbed land (%)		Chann-elization (%)	
	R	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
EPT abundant (%)	–	–	–	–	0.3	0.4	–	–	–	–	–	0.3	–	–	–	–	–	0.4	–	–	–	–	–	–	–	–	–	0.3
EPT abundance (No./sample)[4]	–	–	–	–	–	0.3	–	–	–	–	–	0.3	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	0.3	0.3
EPT taxa (%)[4]	0.3	–	0.3	–	0.4	0.4	–	–	–	–	–	0.3	–	–	–	–	0.3	0.4	–	–	–	–	–	–	–	–	0.4	0.3
EPT taxa (No.)[2,3]	–	–	–	–	0.5	0.5	–	–	–	–	0.3	0.3	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	0.4	0.3
EPT/Chironomid (No., ratio)[3]	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Isopoda abundance (%)	–	–	–	–	0.3	0.3	–	–	–	–	–	–	–	–	–	–	0.4	0.3	–	–	–	–	–	–	–	–	0.3	–
Isopoda taxa (%)[4]	–	0.4	–	–	0.6	0.3	–	–	–	–	0.3	0.3	–	–	–	–	0.3	–	–	–	–	–	–	0.3	–	–	0.5	0.4
Isopoda (No. of taxa)[4]	–	–	–	–	0.5	0.4	–	–	–	–	0.3	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.4	0.4
Oligochaeta abundance (%)[1]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.4	–
Plecoptera abundance (%)	–	–	–	–	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Plecoptera (No. of taxa)[1]	–	–	–	–	0.5	0.4	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Shannon-Wiener diversity index	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	0.3	–
The most abundant taxon (%)[3]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
The other Diptera and non-insect abundance (%)[2]	–	–	–	–	0.4	0.4	–	–	–	–	–	–	–	–	–	–	–	0.4	–	–	–	0.3	–	0.3	–	–	0.3	–
Total number of taxa[1,2,3,4]	–	–	–	–	0.4	0.3	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	0.3	0.3	–	0.3	0.3	–
Tribe Tanytarsini midge (No., %)[2]	–	–	–	–	–	0.3	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–
Tricoptera abundance (%)[2]	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–
Tricoptera (No. of taxa)[1,2]	–	–	–	–	0.5	0.5	–	0.3	–	–	0.3	0.3	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	0.5	0.3
Two most abundant taxa (No. %)[1]	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	0.3	–
Tolerance																												
Depositional abundant (%)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Depositional taxa (%)[4]	–	–	–	–	0.3	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hilsenhoff biotic index[3]	–	–	–	–	0.5	0.3	–	–	–	–	0.3	0.3	–	–	–	–	0.4	0.5	–	–	–	–	–	–	–	–	0.4	0.3
Mean tolerant value[4]	–	–	–	–	0.5	0.5	–	–	–	–	0.3	0.3	–	–	–	–	0.3	0.5	–	–	–	–	–	–	–	–	0.5	0.4
Tolerant organism (No. %)[2]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.4	–

Numbers in superscript indicate metrics used in different indices of biotic integrity (see Table II for explanation). EPT represents members of Ephemeroptera, Plecoptera, and Trichoptera.

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